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MEMORANDUM

SIGNIFICANCE OF THE COLIFORM GROUP OF BACTERIA AND OF OTHER BACTERIA AS A CRITERION OF THE SANITARY QUALITY OF WATER.

By

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Water, the primary essential for life, has been demanded by man from the beginning. Likewise man has demanded that the water be satisfactory. The primary and common criteria for "satisfactory" have been taste, odor and appearance. Through the years, marked advances have been made in the knowledge of the factors concerned in the quality of water and the criteria for "satisfactory" have been modified and extended. The concept of associating water with disease developed long before any scientific knowledge of abnormal physiology. By the middle of the nineteenth century, before the dawn of bacteriology as a science, Snow and Budd in England had associated water with intestinal diseases and had proved definitely that the causative factors were in the water consumed.

The epoch-making studies of Pasteur with his reports of 1858 to 1861 precipitated an intensive investigation in the field of bacteriology. In 1880 Eberth announced the isolation of Bact. typhosis, (now Eberthella typhosa). In 1884 Koch described the comma bacterium as the cause of Cholera. Escherich isolated Bact. coli and aerogenes (now Escherichia coli and Aerobacter aerogenes) from fecal material in 1885. Numerous other studies

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followed and have continued to follow, bringing the science of bacteriology to its present state of knowledge.

In the field of water bacteriology it was natural that the maximum interest should center in the isolation of waterborne intestinal pathogens. As a result, from then until now, considerable effort has been directed to the development of procedures and media for the detection of such intestinal pathogens. To date no medium or procedure has been developed which is adaptable for use in the average laboratory, or which is productive enough to be of value in judging the sanitary quality of waters. Consequently, the detection, quantitatively, of intestinal pathogens in water is a suitable study for research laboratories, and is of great value, not in judging the quality of waters directly, but in establishing a background of information for standards based on other related bacterial genera.

The isolation of Bact. coli and aerogenes by Escherich in 1885, and the demonstration that they were present in normal fecal material in large numbers, indicated the presence of these bacteria as a criterion of the possible presence of intestinal pathogens. Since that time, more work has been done with, and more reports have been issued, regarding this group of bacteria than in any other field of bacteriology. Consequently, no attempt will be made in this brief memorandum to review this very extensive literature, which has been reviewed by others. Rather, the significant facts which have been established regarding criteria of water quality will be pointed out and reference will be limited to citation of particular fields or epochs in the studies.

Recognizing the probable significance of this "B. coli Group" of bacteria in judging the sanitary quality of waters, the American Public

Health Association in 1894 invited a group of American Bacteriologists to appoint a Committee to decide upon procedures for the quantitative determination of these bacteria in water. This Committee, in 1897, presented its first report which later became a part of the First Edition of the Standard Methods for the Examination of Water of the American Public Health Association. Since then, a number of editions of these Standard Methods have been issued, each in turn containing detailed approved procedures (in accord with the development of knowledge at the time) for the determination of the coliform group of bacteria. It should be emphasized, however, that in all instances the procedures given provided for the determination of a group of bacteria, not of any specific genus, regardless of whether the procedures were designated for, (1) B. coli (2) B. coli group, (3) colon group, (4) coli-aerogenes group, or (5) coliform group. In general this included all aerobic and facultative anaerobic Gram negative nonsporeforming bacteria which ferment lactose with gas formation. Moreover, the primary criterion during the past 40 years for estimating the sanitary quality of waters has been this group of bacteria, whether such waters were to be used for drinking, bathing, or other purposes.

As a result of the application of these procedures in the examination of waters over this period, and in connection with epidemiological studies of enteric infections, a very considerable amount of information has been accumulated in regard to the bacteriological quality of safe and unsafe waters. It is upon the foundation of such information that the various bacterial standards governing the sanitary quality of water have been based. At the same time the shortcomings of the current method have been recognized and continuous effort has been directed to the development of a better criterion. It should be noted in passing that if the coliform

group were replaced by some other bacteria or group of bacteria which will be discussed presently then a new foundation of information would have to be obtained upon which new bacterial standards might be based.

In seeking to obtain an improved criterion for indicating the possible presence of enteric pathogens in water, bacteriologists have kept two definite purposes in mind: (1) To utilize media which could be readily prepared and exactly reproduced in the average laboratory. In most instances this difficulty might be overcome by using dehydrated media of a standard brand; and (2) to develop procedures which could be carried on accurately with the personnel and facilities available in the average laboratory. In pursuing these research studies, bacteriologists have followed in general three lines of investigation;

A. To improve and simplify the method of determining the coliform group of bacteria.

B. To select as a criterion organism some bacteria other than those included in the coliform group.

C. To utilize one genus from the coliform group, namely, Escherichia coli.

In studies to improve and simplify methods for the determination of the coliform group the elimination of false positive presumptives has been of primary interest. Toward this end modifications of standard lactose broth by the omission of some ingredients and by the addition of small amounts of toxic agents to inhibit the growth of bacteria other than coliforms has been the major field of study. Brilliant green lactose bile broth, crystal violet lactose broth, fuchsin lactose broth and formate ricinoleate broth may be cited as the major contenders in this field. Ruchhoft (Jour. Amer. W.W. Assn., 27:1732, (1935)), Jordan, (ibid. 24:1027.

(1932)), Salle, (J. Bact. 20:381 (1930)), Ritter, (Jour. Amer. W.W. Assn., 24:413, (1932)), and Stark and England, (J. Bact. 24:26, (1935)) have presented data and reviewed the literature dealing with this use of these media. In general, the finding has been that these media under certain conditions also inhibit the growth of coliforms to such an extent that they are not suitable for use as presumptive media. They did find, however, that some of these media, in particular brilliant green bile lactose broth, are of very great value when used as a confirmatory medium. The literature on such use of these media has been reviewed and data supporting such usage have been presented fully by McCrady, (Amer. Jour. P.H. 27:1243 (1937) and 29:1250 (1939)).

The most recently suggested substitute for Standard lactose broth, Lauryl sulfate tryptose broth (Mallmann and Darby, Amer. Jour. P.H. 31: 127 (1941)), appears to be non-toxic to coliforms and to restrain the growth of non-coliforms, McCrady, (Amer. Jour. P.H., 33:1199 (1943), presents considerable evidence to this end and recommends further studies to determine its value in the examination of finished waters.

The possible use of solid media to obtain plate counts of indicator bacteria, as a substitute for lactose broth, has also been investigated. In this field lactose litmus agar, Ayers and Rupp agar, Brilliant Green Lactose Bile agar (Noble and Tonney, J. Amer. W. W. Assn. 27:108 (1935)), Ferrocyanide Citrate agar, (Tonney and Noble, J. Infec. Dis. 48:413 (1931)), and Methylene Blue Erythrosine Brom Cresol Purple agar (Salle, J. Bact., 20:381 (1930)) have been the principal contenders. These media, and in particular Brilliant Green Lactose Bile agar, have been of major value in research laboratories, but difficulties of preparation and use thus far have rendered them entirely unsuited for routine use in the average laboratory.

B. Streptococci, Bact. pyocyaneus, and anaerobes normally found in the intestinal tract of man have been investigated and tried as indicators of fecal pollution. Although they have been studied extensively in a number of laboratories, in no instance have any of them been adopted as the sole bacterial indicator of water quality. This failure has been due primarily to (1) difficult procedures and apparatus required for the identification of anaerobes, (2) inability to differentiate between fecal and non-fecal streptococci, and (3) the finding that many fecal specimens did not contain pyocyanase organisms at all. As this problem of finding a better indicator organism is of paramount interest, work in this field will undoubtedly be continued.

C. Utilization of Escherichia coli as the indicator organism. It was recognized early, as has been pointed out above, that the characteristics for B. coli of the human intestinal tract as originally described, did not define a pure culture of bacteria, but rather a group of bacteria now called the Coliform Group. Outstanding contributions in the literature to these differential studies are those of Clark and Lubs (Jour. Infec. Dis. 17:160. (1915)), and Levine (Bull. 62, Iowa State College, 1921). Hundreds of other research workers have added their contributions to these studies in efforts to determine the characteristics of the bacteria included in the coliform group so that the ideal bacterial criterion of pollution might be defined. At one time Bergey's Manual of Determinative Bacteriology contained the descriptions of 22 species of the genus Escherichia, 19 of which were designated as of intestinal origin, six species of the genus Aerobacter, three of which were designated as of fecal origin, and a considerable number of coliform group intermediate organisms which were placed in other genera.

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After careful consideration of all of this evidence, the Committee on Standard Methods for the Examination of Water stated in the Eighth Edition of Standard Methods (1936). "The definition of the coli-aerogenes group as given includes organisms of both the so-called fecal and non-fecal types. At the present time, any attempt to evaluate a drinking water on the basis of a distinction between these two types is regarded as unwarranted" As far as is known, no subsequent evidence has been produced which would tend to alter this opinion. As a matter of fact, anyone contemplating such differential procedures would be well advised to read, (1) Sanitary significance of the succession of colon-aerogenes organisms in feces by Parr (Proc. Soc. Exp. Biol. and Med., 32:580 (1935)), in which he concludes "The assumption that organisms of the aerogenes type ~~[illegible]~~ isolated from water, come from grains and soils, and not from feces, is untenable", (2) "The Coliform Bacteria", by Parr, (Bact. Reviews, 3:1-48 (1939)), and (3) "The Incidence of Bact. aerogenes in the faeces of persons suffering from intestinal infections and its significance in Water Analysis", by Krishnan and Chawla, (Ind. Med. Gaz. 76:628 (1941)).

As a suggested guide for continued research in this field the Standard Methods Committee (Standard Methods, 1936) set up three criteria: (1) cultures must be pure (pages 268-9), (2) a minimum of four differential tests, indol production, methyl red reaction, Voges-Proskauer test and growth in citrate, should be applied (pages 269-272), and (3) "when it is desired to differentiate between the coli and the aerogenes sections of the coli-aerogenes group, the detailed procedure shall not follow primary planting in liquid media, but shall be based upon primary planting of the sample in solid media", (page 211). The reason for requirement (3) may be stated as follows. In planting portions of a sample

in liquid media in serial decimal dilutions, each portion of sample yielding a positive result must have contained at least one coliform organism; and actually contained a number of coliforms varying from one to about nine. It has been shown definitely that if this portion of water planted contained one aerogenes and four coli, the one aerogenes may grow so much faster than the four coli that the secondary plate streaked for confirmation will show aerogenes colonies only. Conversely, if this portion of water contained one coli and four aerogenes, the one coli might grow at such a rate that coli colonies only would be found on the confirmatory plate. Ruchhoft et al, (J. Bact. 21:407, (1931) and ibid, 22:125 (1931)), have submitted definite data substantiating these requirements.

Utilizing elevated temperatures instead of toxic chemicals to limit growth to fecal organisms, Perry and Hajna (J. Bact. 26:419, (1933)) have sponsored the use of a modified Eijkman test (Zent. Bakt., I, Orig. 37:742, (1904)), using their E.C. medium with incubation at 45.5°C. ± 0.2°. This procedure has been given considerable study both in this country and abroad. Dodgson as well as Clegg and Sherwood (J. Hyg. Camb. 39:24, (1939)) in England, Ferrarola and Monteverde (Bol. Obras. Sanit., Buenos Aires, 2:248, (1939)), and many workers in this country present data to show that at incubation temperatures above 44° C. (Clegg and Sherwood say that this temperature is critical to ± 0.1° C.) not only the so-called non-fecal coliforms are inhibited but also, particularly at 45.5° to 46° C., a considerable number of true fecal coli. Perry and others are of the opinion that if incubation temperatures more than 0.2° C. below 45.5° C. are permitted, most of the value of the procedure for the elimination of the growth of coliforms other than Esch. coli is lost. Perry and Hajna in their most recent contribution (Amer. Jour. P.H. 34:735 (1944)) presen

the data of a cooperative study by 11 laboratories. The results from five of these cooperating laboratories are in agreement with those obtained by Perry while the data from the other six laboratories were of such a nature that Perry considered them unreliable.

Without resorting to a detailed review of the literature on this subject it appears sufficient for the present purpose to make two observations;

1). On theoretical grounds the optimum temperature for growth of fecal coliforms would be 37° C., (98.6° F.), while that of the so-called non-fecal members of the group would be lower. Aerogenes types and intermediate forms common to the intestinal tract of man should have the same optimum temperature as fecal coli. It is known that growth at a retarded rate occurs through a wide range when incubation temperatures are reduced below the optimum. As incubation temperatures are elevated above the optimum, growth is first stimulated and then retarded as the temperature increases, until at some point growth ceases and at a higher zone death of the cells occur. It would not be expected that all of the cells of a given bacterial species present in a portion of water, would react in the same manner or be similarly affected by exactly the same degree of temperature. Depending on the physical condition of the cell as induced by natural or artificial purification processes, the critical temperature zone for stasis of fecal coliforms might well vary between 44° and 46° C. (111.2° and 114.8° F.) or even between 42° and 46° C., and for an accurate determination of fecal coliforms in an average sample, under certain conditions, even 44° C. might be too high an incubation temperature.

2). In the report of Perry and Hajna referred to above, 11 laboratories cooperated in the study. Presumedly, from the information gi[illegible]

in the report all laboratories used the same dehydrated media and their methods and procedures were coordinated by the proponent of the medium and procedures. Yet only five of 11 cooperating laboratories obtained results which the proponent considered reliable. Under such conditions it does not appear reasonable to assume that this medium and procedure are suitable for adoption in Standard Methods for use in the average laboratory. In this connection, it should be noted that if new bacteriological criteria for judging the quality of waters were adopted, then new standards, based on new foundation information, would be required also. In the case of the Esch. coli section of the coliform group, an educated guess might be made as to the new standard. That is, if the method used for the determination of Esch. coli were readily applicable and productive, it could be assumed that the new standard should be about twice as severe as the one established for the coliform group, as in studies which have been made the Esch. coli section represent about one-half of the coliform organisms present in normally polluted waters. If the method of determination of Esch. coli were not productive, then the new standard would need to be still more stringent to allow for the failures of the method.

Thus it appears that while it is desirable to seek for better bacterial criteria of pollution in water, and these researches should be continued, the results of such studies to date have not produced a criterion which may be considered to have satisfactorily replaced the coliform group. The qualifications of the coliform group to serve in this capacity may be summarized as follows:

1). Bacteria of the coliform group are invaribly found in large numbers in the feces of man and certain animals. This applies to all members of the group. Studies have shown that the average number of such

organisms excreted per capita per day is about, (a) 30,000 billion when based on the examination of fresh feces, and (b) 12,000 billion when based on the examination of domestic sewage. In making this point it should be noted that members of the aerogenes section of the coliform group are also found in relatively large numbers in, and on, certain unpolluted grains and vegetables. However, organisms from such sources are few as compared with the large numbers found in fresh feces and their presence would confuse an interpretation of water quality only in areas entirely free from possible pollution with feces from human or animal sources. Such areas are practically non-existent where water quality is a matter of concern.

2). Analysis of the records for water quality as based on the coliform group, and for intestinal diseases in communities with public water supplies during the past 20-30 years, indicates that the coliform group has served effectively as an indicator of fecal pollution when the procedures used for the determination of the coliform group were carried out in accordance with Standard Methods. Kehr and Butterfield (Public Health Reports, 58:589 (1943) Reprint No. 2469), using available data, have attempted to show the relation existing in water between coliform, enteric pathogens and intestinal diseases.

3). All component members of the coliform group of bacteria tend to die out in waters at approximately the same rates regardless of whether the exposure is to natural or artificial purification processes. Buchhoft et al., (J. Bact. 25:143 (1933)), have presented data to definitely establish this point. In this connection it should be pointed out that Buchhoft in this study, followed the correct procedure to obtain accurate and reliable data, namely, all primary plantings of samples were made in solid media.

4). A method (primary planting in lactose broth with confirmation in Brilliant Green Lactose Bile broth) is available for the determination of the coliform group which is, (a) as accurate as the Standard Methods Completed Test, (b) relatively simple so that it may be carried out in the average laboratory without error in procedure or in interpretation, and (c) approved by the U.S.P.H.S. Drinking Water Standard (Public Health Reports, 58:63, (1943) Reprint No. 2440), and will be approved (personal communication of the Chairman of the Committee) for the examination of raw and finished waters in the forthcoming Ninth Edition of Standard Methods for the Examination of Water, of the American Public Health Association.